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**REVIEWER'S REPORT**

**Manuscript No.:** JNAVES-016

**Title: DEVELOPMENT OF AG NANOPARTICLE BIOCHAR EMBEDDED PVA/ALGINATE BEADS AND THEIR APPLICATION AGAINST GRAM-POSITIVE BACTERIA.**

**Recommendation:**
Accept as it is .....
**Accept after minor revision**.....
Accept after major revision .....
Do not accept (*Reasons below*) .....

**Reviewer's ID: JPRAVES-052**

| Rating | Excel. | Good | Fair | Poor |
|---|---|---|---|---|
| Originality | | ✓ | | |
| Techn. Quality | | | ✓ | |
| Clarity | | ✓ | | |
| Significance | | ✓ | | |

## *Detailed Reviewer's Report*

### General Comments

This manuscript presents the development of silver nanoparticle-loaded biochar embedded within polyvinyl alcohol/sodium alginate (PVA/SA) hydrogel beads and evaluates their antibacterial activity against gram-positive bacteria. The study addresses a relevant topic in biomaterials and environmental sustainability by utilizing agricultural waste (corn husk) as a biochar precursor and employing a green synthesis approach for silver nanoparticle production. The work demonstrates potential applications in antibacterial and biomedical fields. However, the manuscript requires significant revisions to improve scientific rigor, data presentation, methodological clarity, and language quality before it can be considered for publication.

### Content and Originality

**Strengths**

- The utilization of corn husk-derived biochar as a support material for silver nanoparticles is environmentally attractive and contributes to waste valorization.
- The use of tea polyphenols as a green reducing agent aligns with sustainable synthesis principles.
- The focus on gram-positive bacteria (Staphylococcus aureus and Staphylococcus epidermidis) adds value since many previous studies emphasize gram-negative bacteria.
- The study combines structural characterization, swelling behavior, mechanical properties, and antibacterial evaluation in a single material system.

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**Concerns**

1. The novelty of the work is moderate rather than highly innovative, as AgNP-loaded biochar composites and PVA/alginate hydrogel systems have been extensively reported.
2. The manuscript does not clearly explain how the developed material substantially advances beyond previously reported Ag/biochar/PVA-alginate systems.
3. The comparison with previous studies in Table 1 is limited and should include quantitative performance comparisons.
4. The biomedical relevance is claimed repeatedly, but no cytotoxicity or biocompatibility evaluation is provided.

**Technical Quality**

**Minor Revision Required**

1. **Insufficient Experimental Details**
   - The preparation procedure lacks information regarding bead size distribution, reproducibility, and batch consistency.
   - The concentration of Ag nanoparticles deposited onto biochar is not adequately quantified during synthesis.
2. **Limited Characterization**
   - No XRD analysis is presented to confirm silver nanoparticle formation and crystallinity.
   - TEM analysis is absent, preventing visualization of nanoparticle size and distribution.
   - SEM images were collected at only 120× magnification, which is insufficient to evaluate nanoparticle morphology.
3. **Antibacterial Assessment**
   - Only zone of inhibition tests were performed.
   - No minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC) data are provided.
   - The study lacks statistical analysis of antibacterial results.
   - Antibacterial performance is evaluated against only two bacterial strains.
4. **Mechanical Testing**
   - Compressive strength data are reported without standard deviations or replicate measurements.
   - Statistical significance between PVA/SA and PVA/Ag-BCH/SA samples is not demonstrated.
5. **Swelling and Stability Evaluation**
   - Error bars are presented, but the number of replicates is not stated.
   - Long-term stability is only visually assessed after immersion.
   - Silver ion release behavior is not investigated despite its importance for antibacterial activity.

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6. **Data Interpretation**
    - Several conclusions regarding silver distribution and antibacterial mechanisms are speculative and not directly supported by experimental evidence.
    - Claims regarding biomedical applications are premature without toxicity assessment.

**Language and Presentation**
**Minor Revision Required**
The manuscript contains numerous grammatical and stylistic issues that require professional English editing.
Examples include:

- Incorrect sentence structures and tense inconsistencies.
- Repetitive wording throughout the Results and Discussion section.
- Several awkwardly constructed sentences affecting readability.
- Inconsistent formatting of units, symbols, and scientific terminology.

Additional concerns:

- Figure captions require improved detail.
- Some figures have low resolution and lack sufficient labeling.
- Scientific notation and superscripts/subscripts should be standardized throughout the manuscript.

**Structure and Organization**
**Strengths**

- The manuscript follows a conventional scientific format.
- Objectives are clearly stated in the Introduction.
- Results generally follow the order of experimental procedures.

**Concerns**

1. The Introduction is overly lengthy and contains repetitive discussion.
2. The Results and Discussion section is largely descriptive and lacks deeper scientific interpretation.
3. Mechanistic explanations should be consolidated and strengthened.
4. The manuscript lacks a dedicated limitations section.
5. Future research directions should be discussed more comprehensively.

**References and Citations**
**Minor to Moderate Revision Required**
**Strengths**

- References are generally relevant and support the study background.
- Several recent publications are included.

**Concerns**

1. The reference list is relatively small for a research article in this field.
2. Additional recent studies on AgNP-loaded hydrogel systems should be incorporated.

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3. Some citations are used repeatedly while broader literature coverage is limited.
4. The manuscript should include more references concerning:
    - Silver nanoparticle release mechanisms.
    - Cytotoxicity and biomedical safety.
    - Advanced antibacterial hydrogel materials.
5. Reference formatting should be checked carefully for journal consistency.

**Overall Recommendation**

The manuscript presents an environmentally relevant and potentially useful antibacterial composite material based on agricultural waste-derived biochar and silver nanoparticles. The experimental findings indicate successful preparation of PVA/Ag-BCH/SA composite beads with enhanced antibacterial performance and improved swelling resistance. However, significant methodological and analytical shortcomings limit the scientific impact of the work. Additional characterization, stronger antibacterial evaluation, statistical validation, and improved discussion are necessary to support the conclusions.

**Final Decision:**
**Minor Revision**